

Delayed effects of severe hypoxia experienced by marine gastropod embryos

C. J. Segura^{1,*}, O. R. Chaparro¹, J. A. Pechenik², K. A. Paschke³, S. J. A. Osoro¹,
J. M. Navarro¹, V. M. Cubillos¹

¹Instituto de Ciencias Marinas y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia 5090000, Chile

²Biology Department, Tufts University, Medford, MA 02155, USA

³Instituto de Acuicultura, Universidad Austral de Chile, Casilla 1327, Puerto Montt 5480000, Chile

ABSTRACT: Although periods of severely reduced oxygen concentration are occurring with increasing frequency in coastal marine ecosystems, the effects of such exposures on early development have not been extensively explored. Brooding females of the gastropod *Crepipatella dilatata* expose their embryos to such hypoxic conditions ($<1 \text{ mg O}_2 \text{ l}^{-1}$) naturally during periods of intense seasonal rains, when they isolate their brood chambers from the external environment for long periods. We investigated the effects of such reduced oxygen availability on the encapsulated embryos and also looked for delayed ('latent') effects on juveniles after their emergence from the mothers. After 24, 48 or 72 h at oxygen levels $<1 \text{ mg O}_2 \text{ l}^{-1}$, at 12°C and 30 psu salinity, females were transferred to aerated seawater. Many females selectively evicted some of their egg capsules, particularly those containing advanced embryos. Also, juvenile emergence was delayed, although those individuals emerging later had the same mean size as those derived from non-stressed embryos. Latent effects were also observed: the hypoxia experienced during embryonic development reduced the number of juveniles successfully emerging per female and severely compromised juvenile growth and survival over the next 30 d. Our results show that extended periods of severe hypoxic conditions can have a dramatic impact on early development and reproductive fitness, with some effects not appearing until long after normoxic conditions have been restored and the juveniles have emerged into the natural environment. The study also shows that brooding can in some situations expose embryos to severe stresses, even while protecting embryos from other stresses.

KEY WORDS: Hypoxia · Brooding · Latent effects · *Crepipatella*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Many marine animals from a variety of phyla brood their embryos for a time, typically using specialized internal or external body parts (fish, Jones & Reynolds 1999, Wilson et al. 2003; echinoderms, Hendler 1975; polychaetes, Blake et al. 2009; crustaceans, Baeza & Fernández 2002, Vogt 2013; bivalves, Chaparro et al. 2011a, Tankersley & Dimock 1993; gastropods, Chaparro et al. 2005a). Such behavior is generally and understandably considered to be pro-

tective (Gillespie & McClintock 2007) against both predators and external environmental stresses (Chaparro et al. 2008b). However, under some conditions, brooding can expose embryos to deleterious conditions within the brood chamber even while protecting them from deleterious external conditions. Some gastropod and bivalve species, for example, brood their embryos in the mantle cavity, where the gills are also situated (Chaparro et al. 2001, 2008c, Castanos et al. 2005, Ituarte 2009). As a response to desiccation or salinity stress, such animals typically use

their shells as barriers: bivalves can simply close the shell valves together, while gastropods can adhere tightly to the substrate, in both cases thereby isolating the interior brood chamber/mantle cavity from the external environment (Hand & Stickle 1977, Shumway 1977, Djangmah et al. 1979, Berger & Kharazova 1997, Chaparro et al. 2009a, 2011b, Montory et al. 2009, Sussarellu et al. 2012). Although such behaviors can maintain high salinities in the molluscan mantle cavity for long periods of time, despite greatly reduced external salinities (e.g. Chaparro et al. 2009a), water quality within the mantle cavity, where the embryos are brooded, gradually deteriorates (Chaparro et al. 2009a). Such deterioration can include severely decreased concentrations of dissolved oxygen (Chaparro et al. 2009a, Segura et al. 2010). Although the effects of hypoxia on adult physiology and behavior have been reasonably well explored for a variety of organisms in recent years (Tankersley & Dimock 1993, Christensen & Colacino 2000, McMahon 2001, Wu 2002, Gorr et al. 2010, Liu et al. 2011, Connor & Gracey 2012, Wang et al. 2012), few studies have considered the effects of hypoxia on early development, and only one (Li & Chiu 2013) has looked for latent effects—effects that appear well after the stress has ended (reviewed by Pechenik 2006).

During seasonal periods of intense spring rains, brooding females of the suspension-feeding marine gastropod *Crepidula dilatata* (Lamarck, 1822) completely isolate their brood chambers from the external environment (Chaparro et al. 2009a,b, Montory et al. 2009) for 72 h or more whenever the salinity falls below 22 psu (Chaparro et al. 2009a), generating severely hypoxic conditions within the brood chamber (Chaparro et al. 2009a). Indeed, because the chamber in which the embryos are brooded has such a small volume (~1 to 2 ml, Chaparro et al. 2011b), the fluid surrounding the brooded embryos of *C. dilatata* becomes severely hypoxic (<1 mg O₂ l⁻¹) after being isolated from the external environment for only a few hours (Chaparro et al. 2009a). Unfortunately for the embryos of this species, such periods of intense rainfall coincide with peak reproductive activity in our study area, the Quempillén estuary, Chile (C. J. Segura pers. obs.), a place that exhibits ambient salinities as low as 6 psu during this time (Toro & Winter 1983). The gastropod *C. dilatata* thus provides an excellent system for studying the consequences of hypoxia in early development.

Several recent studies have considered the immediate impact of hypoxia on embryonic and larval survival for a number of marine invertebrate species

(Eerkes-Medrano et al. 2013, Leung et al. 2013). We also know that exposure to hypoxic conditions causes females of *C. dilatata* to evict at least some of their egg capsules (C. J. Segura unpubl. data). However, the possibility that hypoxia experienced by brooded embryos may affect the embryos in more subtle ways, well after the stress has ended, has not previously been considered. Certainly, some stresses experienced by free-living larvae often have such negative long-term effects (called 'latent effects,' Pechenik 2006) much later in development; this has been particularly well documented for larvae of marine invertebrates (Pechenik et al. 1998, Qiu & Qian 1999, Pechenik & Rice 2001, Phillips 2002, 2004, Pechenik 2006, Thiyagarajan et al. 2007, Chiu et al. 2008, Li & Chiu 2013). For example, exposing the planktotrophic larvae of some marine animals to reduced food availability, thermal stress, or low salinity for just a portion of the larval stage can compromise future juvenile survival, reduce juvenile growth rates, and delay reproductive maturity (Miller 1993, Pechenik et al. 1996a,b, Qiu & Qian 1999, Pechenik & Rice 2001, Phillips 2002, 2004, Thiyagarajan et al. 2007, reviewed by Pechenik 2006). Moreover, Li & Chiu (2013) recently found that, at least under some conditions, exposing the free-living larvae of *Crepidula onyx* to low oxygen for 8 to 10 d prior to metamorphosis reduced juvenile growth rates significantly in normoxic conditions.

In the present investigation, we sought (1) to understand the relationship between the amount of time that brooding females of *C. dilatata* (and their brooded egg masses) were exposed to low oxygen availability and the likelihood of egg capsule eviction; (2) to determine whether capsules are evicted selectively or at random with regards to number of embryos per capsule; (3) to document any differences in susceptibility to hypoxia for embryos at different stages of development; and (4) to determine whether exposing brooded embryos to low oxygen for 24 to 72 h can produce latent effects on emergence times, juvenile growth rates, or juvenile survival well after the stress has ended.

We believe this to be the first study to consider the potential longer-term impact of hypoxia experienced by brooded embryos. Although our study focuses on a single gastropod species, we believe that our results have far broader implications, as hypoxic conditions have become more common in freshwater and coastal environments around the world, due to climate changes and increasing input of anthropogenic nutrients and organic matter (Wu 2002, Levin 2003, Matear & Hirst 2003, Diaz & Rosenberg

2008, Middelburg & Levin 2009, Howarth et al. 2011, Ho & Burggren 2012, Zhang et al. 2013).

MATERIALS AND METHODS

The study organism

The gastropod *Crepidatella dilatata* is especially well-suited for these studies. Females enclose their fertilized eggs within a group of balloon-like, thin-walled egg capsules, with hundreds of eggs contained within each capsule. A variable number of nurse eggs are enclosed within each egg capsule as a food source for the developing embryos (Chaparro et al. 1999). Females attach the stalks of the capsules to the hard substrate (especially rocks and shells) on which they live and shelter those capsules from predators and other environmental stresses within the mantle cavity beneath the shell (Chaparro et al. 2008a). Within this cavity, the capsules are bathed by the mantle fluid for at least 18 d (Chaparro & Paschke 1990), until the offspring hatch from the capsules and then emerge into the adult population as juveniles (Gallardo 1979, Chaparro et al. 2008a). During the incubation period, the females of this species at our study site (the Quempillén estuary) are exposed to severe and prolonged reductions in salinity during seasonal periods of heavy rains that overlap with this species' peak breeding period (Chaparro et al. 2008b,c). Previous studies have shown that adults of *C. dilatata* isolate themselves from the external environment whenever ambient salinity falls to 22 psu (Chaparro et al. 2008b, 2009a), a condition that lasts for up to 72 h in the Quempillén estuary during the spring breeding season.

Obtaining brooding females and staining embryos with calcein

During the reproductive period, 50 females of *C. dilatata* with shell lengths of 3.0 ± 0.5 cm (mean \pm SD) were collected each week from the Quempillén river estuary ($41^\circ 52' S$, $73^\circ 46' W$; southern Chile), to determine the percentage of brooding females in the population over time. When $>70\%$ of the females were brooding, we collected about 400 individuals of 3.0 ± 0.5 cm shell length; all were attached to empty shells of the bivalve *Mytilus chilensis*. A hole was drilled into each *Mytilus* shell valve with a 2 mm diameter drill bit to connect the external environment with the internal brooding environment of the female pallial

cavity. Thus we made sure that the brooded embryos were exposed to the conditions of the external environment despite any possible attempts of brooding females to isolate the pallial cavity in response to hypoxic conditions.

Before the experiments, 400 females were maintained for 7 d in aquaria ($360 \times 40 \times 25$ cm) with flowing seawater (salinity > 28 psu) at constant temperature ($12 \pm 1^\circ C$) and with continuous aeration (approx. $8 \text{ mg O}_2 \text{ l}^{-1}$). Immediately before beginning the experiments, all females were exposed for 4 h to a solution of calcein (2,4-bis-[N,N'-di(carbomethyl)-aminometil]-fluoresceina, Sigma #C0875) at 100 mg l^{-1} , to mark the shells of the incubated embryos (Moran 2000, Montory et al. 2009). Such staining was possible because females pumped seawater with high salinity containing the calcein to the interior of the pallial cavity, where the embryos were brooded. The staining of embryos allowed us to determine whether or not the embryos had shells at the start of each experiment, and if they did, to determine their shell lengths at the start of the study, all without our having to disrupt the brooding process. Females were then transferred into another aquarium containing seawater at a salinity > 28 psu. At the end of the experiment all juvenile shells were collected and examined using a fluorescent microscope. If the shells showed the calcein mark under the fluorescent microscope, then shell lengths were quantified to identify the stage and size at which organisms had been exposed to hypoxia. Shell length measurements were carried out using digital image processing software (QCapture Suite V. 2.98.2). Juveniles lacking a calcein mark in their shell at emergence were considered to have been non-shelled embryos when they had been exposed to hypoxia. Depending on the developmental stage or shell length of embryos at the beginning of the experiments, embryos were assigned to one of the following categories: (1) early embryonic stage (embryos without shell, diameter $< 300 \mu\text{m}$); (2) veliger intermediate stage (shell length 400 to 799 μm , with velum well defined); and (3) advanced pre-hatching stage (close to hatching, shell length $> 800 \mu\text{m}$, foot well developed).

Exposing brooded embryos at different developmental stages to severe hypoxia

These studies were conducted using a single non-recirculating aquarium containing 70 l of seawater (salinity > 28 psu) at constant temperature ($12^\circ C$). Oxygen concentration was reduced to levels of

severe hypoxia ($<1 \text{ mg O}_2 \text{ l}^{-1}$) by bubbling N_2 gas into the seawater. To maintain desired low levels of oxygen throughout the exposure period, we bubbled nitrogen into the seawater continuously. Dissolved oxygen concentrations were monitored with optic technology using a Presens Microx TX3 micro-sensor. The sensor was previously calibrated in anoxic water using a saturated solution of Na_2SO_3 , and in oxygen-saturated water by bubbling air constantly into the water. Immediately after the calcein stain was applied, 100 control females were obtained at random and placed in a series of small individual containers ($7 \times 5 \times 4 \text{ cm}$) with mesh forming the top and bottom surfaces to allow for ample water flow and to retain any evicted egg capsules or emerged juveniles. These containers were then placed into a single large aquarium tank supplied with flowing seawater, so that all females were held under the same conditions. The microalgae *Isochrysis galbana* was added as supplementary food.

The remaining calcein-stained snails ($N = 300$) were exposed to severe hypoxia for 24, 48 or 72 h in a different large, common aquarium with the same dimensions as described in the previous section. At the end of each exposure, approximately 100 brooding females were randomly chosen from the common aquarium and moved to small individual mesh-sided containers, as described in the paragraph above, and placed in the same well-aerated common aquarium (70 l, running seawater, 12°C , food supplemented with *I. galbana*). Females were then allowed to continue brooding. We checked daily for capsule eviction and for emergence of juveniles as described below.

Early impact of restricted oxygen on brooded embryos

Any impact of severely reduced oxygen availability on females and their brooded embryos prior to hatching was considered to be an 'immediate effect'. We measured the following immediate effects:

(1) Time until egg capsules were ejected ('evicted') from the mantle cavity and the percentage of females ejecting at least some capsules: For 76 brooding females held individually, we recorded the time from the end of the stress until each eviction process began and ended. The percentage of brooding females evicting any of their egg capsules was determined.

(2) Embryonic packaging within evicted egg capsules: From each evicting female (control = 0 evicting females, 24 h = 21, 48 h = 22, 72 h = 33 evicting

females), we recorded the total number of capsules evicted during the brooding period. Evicted capsules were then carefully opened and digital images of their contents were obtained using an Olympus SZ61 stereomicroscope coupled with an image processor system (includes Q-Imaging MicroPublisher 5.0 digital camera and QCapture Suite V. 2.98.2 image analysis software). These images were used to determine the number of embryos in each evicted egg capsule, and to record the stages of embryonic development.

(3) Size of embryos: Embryos from each evicted capsule (total evicting females: $N = 76$) were placed in an Eppendorf tube and preserved in a solution of 80% ethanol and 20% filtered seawater. Embryo diameters (for early embryos) and shell lengths (for later-staged veligers) were later measured at $40\times$ magnification. For embryos that were shelled at the time of exposure to the stress, the calcein marks in the shells were identified using an Olympus microscope (model BX41) equipped with a mercury lamp (model USH-1030L, 100 W bulb) and epi-illumination via a blue light filter (wavelength centered at 460 nm). The calcein mark allowed us to determine whether or not embryos had shells when they were exposed to the hypoxic conditions, and if so, what the shell length was at the time of exposure. This allowed us to later determine the relationship between degree of exposure to stress and the size and stage of development at which evicted embryos had been stressed.

Latent effects on early juvenile

We measured time to emergence and number of juveniles released per female. Shell length and juvenile survival were quantified at 30 d after emergence to determine whether exposing encapsulated embryos to severe hypoxia had latent effects on subsequent development. We collected the following data:

(1) Time until emergence of juveniles from the brooding mother ($N = 325$) and the number of juveniles released per female ($N = 325$): We determined the length of time between exposing the mothers to severe hypoxia and the day on which the first juveniles emerged into the adult population, as well as the number of juveniles released per female. Emergence times were examined as a function of the degree of hypoxic stress experienced and the developmental stages at which they had received the hypoxia stress during the brooding period.

(2) Juvenile shell length and survival at 30 d after emergence from the female ($N = 325$): 4 to 5 juveniles

were collected per female and preserved at 30 d post emergence. Shells of these juveniles were observed using an epifluorescence microscope to identify the calcein mark on the shell, enabling us to determine the size at which individuals had been exposed to the stress. Additionally, photographs taken of each specimen allowed us to measure shell lengths. The average values from the 4 to 5 juveniles that were studied from each female were used for the statistical analyses.

When juveniles were 30 d old, survival was determined by comparing the number of juveniles released from each female on Day 0 with those remaining alive after 30 d.

Statistical analysis

Normality and homoscedasticity of data were tested before further analysis. When those assumptions were met, we analyzed the data using ANOVA, with a significance level of $p < 0.05$. A 1-way ANOVA was used to identify whether the mean initial sizes (embryo diameter or shell length) of the 3 developmental stages used in the study (early embryo, intermediate veliger, advanced pre-emergence individuals) were significantly represented in each hypoxia treatment previous to the start of the experiment using embryos from 325 females. A 2-way ANOVA was used to identify the effects of hypoxic stress for embryos in different stages of development on time until egg capsule eviction, number of capsules evicted, and number of embryos per evicted egg capsule.

Data on time to eviction and percentage of evicting females were square-root transformed before analysis to meet the assumptions of the statistical tests used. When significant differences were identified, we then used an *a posteriori* Tukey test to determine the source of those differences.

A non-parametric chi-square test was carried out on data concerning the effects of hypoxia on the percentage of females evicting egg capsules at each development stage in comparison with the control results.

Data on size at emergence, juvenile shell length, number of emerged embryos per female, and mean juvenile survival per female were examined for latent responses to hypoxia. Statistically significant differences were quantified by 2-way ANOVA, considering as factors the stage of embryonic development and the duration of exposure to hypoxia to identify the impact that hypoxia experienced as embryos at different developmental stages had on

subsequent juvenile development. Data for juvenile size at emergence and juvenile shell length at 30 d after emergence were natural log transformed to fulfill the assumptions of the ANOVA. A Tukey multiple comparison *a posteriori* test was used to identify significant differences at a level of $p < 0.05$.

RESULTS

Immediate impacts of hypoxia on egg capsule eviction

Embryos used in the hypoxia experiments fell into 1 of 3 groups (early pre-shelled, intermediate veliger and advanced near hatching individuals) differing significantly in initial size (1-way ANOVA, $F_{(2,325)} = 406.16$, $p < 0.001$).

Control females did not evict any egg capsules during the study, in contrast to females exposed to hypoxic events (Fig. 1). The time-lag before any capsules were evicted, the percentage of females evicting, the number of capsules evicted per female and the number of embryos per evicted capsule all varied with the duration of the hypoxic stress. Specifically, capsule eviction (all capsules considered together, without considering stage of development) was initiated more quickly after longer exposure to the hypoxic stress (Fig. 1, Table 1), and females brooding more advanced embryos evicted their egg capsules sooner than those brooding capsules containing

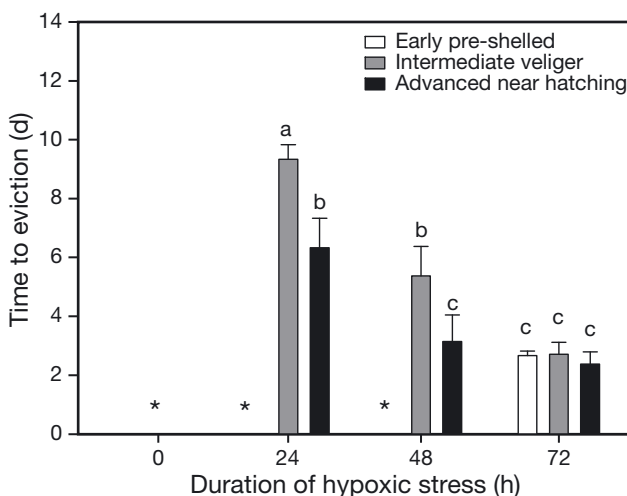


Fig. 1. *Crepipatella dilatata*. Influence of the duration of hypoxic stress and stage of embryonic development on mean time to egg capsule eviction by females following exposure to hypoxia ($<1 \text{ mg O}_2 \text{ l}^{-1}$) for up to 72 h ($N = 73$). Vertical bars represent SE. Different letters above the bars represent significant differences between means. * = no capsules were evicted

intermediate stage embryos (Table 1) (e.g. average time to eviction varied from 9.3 d for females brooding intermediate veligers stressed for 24 h, to only 2.7 d for those stressed for 72 h, Fig. 1).

For all levels of hypoxic stress (24, 48, or 72 h), more females evicted egg capsules if the stress had been experienced by intermediate and advanced prehatching stages rather than by individuals at earlier stages of development. The percentage of females evicting their egg capsules was highest when they had been brooding advanced embryos (Fig. 2). Females brooding embryos in early stages of development evicted capsules only after being subjected to the most intense level of stress (Fig. 2).

The total number of capsules eliminated by evicting females increased significantly with the duration of exposure to the stress (Fig 3, Table 1). However, the number of capsules evicted following each hypoxia treatment was not related to the stage of embryonic development at the time that stress was experienced (Fig. 3, Table 1). Females exposed to hypoxic stress for 24 h evicted significantly fewer capsules, on average, than those exposed for 48 h (7 vs. 11 capsules per female, respectively), while a 72 h exposure did not produce a significantly greater response than that produced by a 48 h exposure (Fig. 3, Table 1). The mean number of embryos inside evicted capsules increased significantly with duration of exposure to the hypoxic stress (Fig. 4, Table 1). However, stage of development when stressed had no significant impact on the number of embryos found in evicted egg capsules (Fig. 4, Table 1). But again, for each developmental stage at the time of exposure, the number of embryos per evicted egg capsule increased with longer exposure to the stress. For example, females brooding intermediate-stage shelled embryos evicted egg capsules containing an average of 13 embryos each following 24 h exposure to severe hypoxia, while those

Table 1. *Crepipatella dilatata*. Two-way ANOVA for the effects of the duration of hypoxic stress (0, 24, 48 and 72 h) and the stage of embryonic development (early pre-shelled, intermediate veliger and advanced near hatching) on the time to eviction, number of evicted capsules per female, evicted embryos per capsule, time to emergence, number of emerged juveniles per female, size at emergence, juvenile shell length (30 d after emergence) and juvenile survival per female (30 d after emergence). **Bold** p-values indicate statistical significance

Source	df	MS	F	p	Figure
Time to eviction					
Hypoxic stress	2	152.64	55.92	0.0001	Fig. 1
Stage embryos	1	55.06	20.17	0.0001	
Stress × Stage	2	11.15	4.08	0.021	
Error	67	2.72			
No. of evicted capsules per female					
Hypoxic stress	2	82.87	6.39	0.003	Fig. 3
Stage embryos	1	1.98	0.15	0.697	
Stress × Stage	2	2.23	0.17	0.842	
Error	67	12.96			
Evicted embryos per capsule					
Hypoxic stress	2	330.50	11.92	0.0001	Fig. 4
Stage embryos	1	45.28	1.63	0.206	
Stress × Stage	2	27.14	0.97	0.381	
Error	67				
Time to emergence					
Hypoxic stress	3	965.49	649.15	0.0001	Fig. 5
Stage embryos	2	1058.72	711.83	0.0001	
Stress × Stage	6	23.77	15.98	0.0001	
Error	313	1.48			
No. of emerged juveniles per female					
Hypoxic stress	3	7450.19	21.52	0.0001	Fig. 6A
Stage embryos	2	92.72	0.26	0.76	
Stress × Stage	6	308.75	0.89	0.50	
Error	313	346.16			
Size at emergence					
Hypoxic stress	3	0.06	10.79	0.67	Fig. 6B
Stage embryos	2	0.002	0.31	0.73	
Stress × Stage	6	0.004	0.63	0.70	
Error	313	0.006			
Juvenile shell length					
Hypoxic stress	3	5.74	338.20	0.0001	Fig. 7A
Stage embryos	2	0.004	0.21	0.80	
Stress × Stage	6	0.01	0.57	0.74	
Error	313	0.02			
Juvenile survival per female					
Hypoxic stress	3	23 009.2	162.68	0.0001	Fig. 7B
Stage embryos	2	14.21	0.10	0.90	
Stress × Stage	6	190.24	1.34	0.23	
Error	313	141.43			

stressed for 72 h evicted egg capsules containing an average of 23 embryos each (Fig. 4). Note that no females evicted any egg capsules following 24 or 48 h exposures to stress if the capsules had contained early pre-shelled embryos at the time of exposure (Fig. 4).

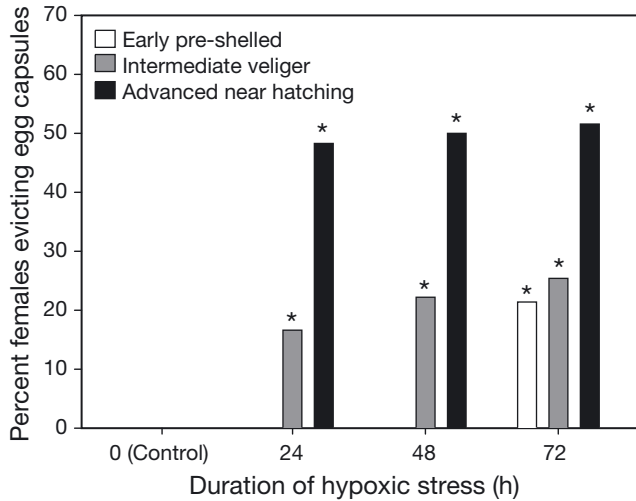


Fig. 2. *Crepipatella dilatata*. Influence of exposure to hypoxia for up to 72 h on egg capsule eviction by brooding mothers. * = significant differences from control treatments within each stage of development (χ^2 , $p < 0.0001$). Control females, N = 76; females used in 24 h exposures, N = 75; females used in 48 h exposures, N = 74; females used in 72 h exposures, N = 100

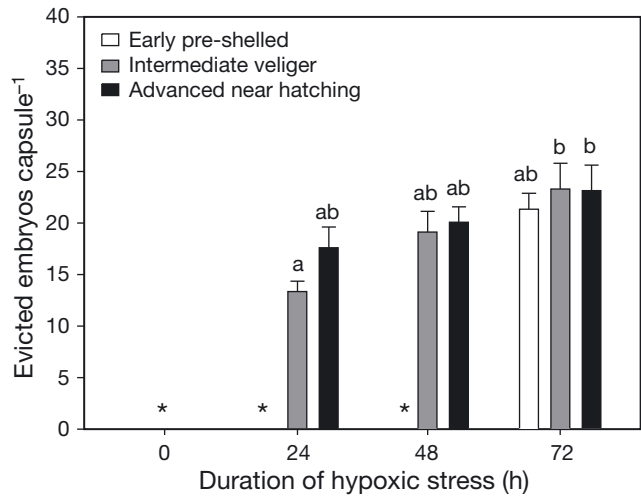


Fig. 4. *Crepipatella dilatata*. Influence of embryonic stage and duration of hypoxic stress on the number of embryos per evicted egg capsule following exposure to hypoxic stress (N = 73). Vertical bars represent SE. Different letters above the bars represent significant differences between means. * = no capsules were evicted

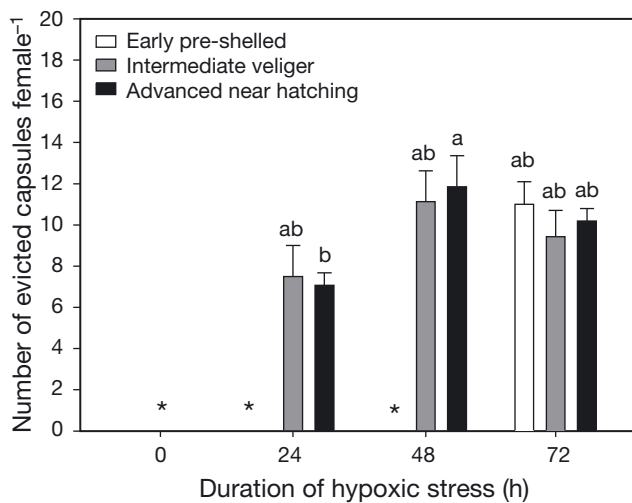


Fig. 3. *Crepipatella dilatata*. Influence of the duration of hypoxic stress and embryonic stage of development on the number of egg capsules evicted by brooding females exposed to hypoxia for 0 to 72 h (N = 73). Vertical bars represent SE. Different letters above the bars represent significant differences between means. * = no capsules were evicted

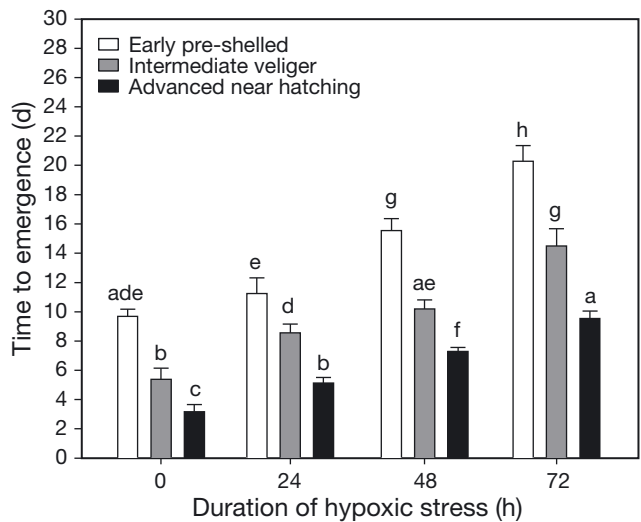


Fig. 5. *Crepipatella dilatata*. Influence of stress duration and embryonic stage at time of exposure on time to juvenile emergence following exposure of brooding females to hypoxia for the times indicated (total females N = 325). Vertical bars represent SE. Different letters above the bars represent significant differences

Latent effects due to oxygen restriction during brooding

Emergence from maternal protection was delayed following prolonged exposure to the stress (Table 1); the magnitude of the effect varied inversely with embryonic stage of development at every stress level (Table 1). Specifically, time to emergence was de-

layed for about 10 d for embryos experiencing the maximum stress (72 h) in early development, by about 9 d for those stressed as intermediate veligers, and for only about 6 d for those stressed as advanced embryos, in comparison with controls (Fig. 5).

The number of juveniles released per female decreased with increased exposure to severe hypoxic conditions (Fig. 6A, Table 1); the effect was inde-

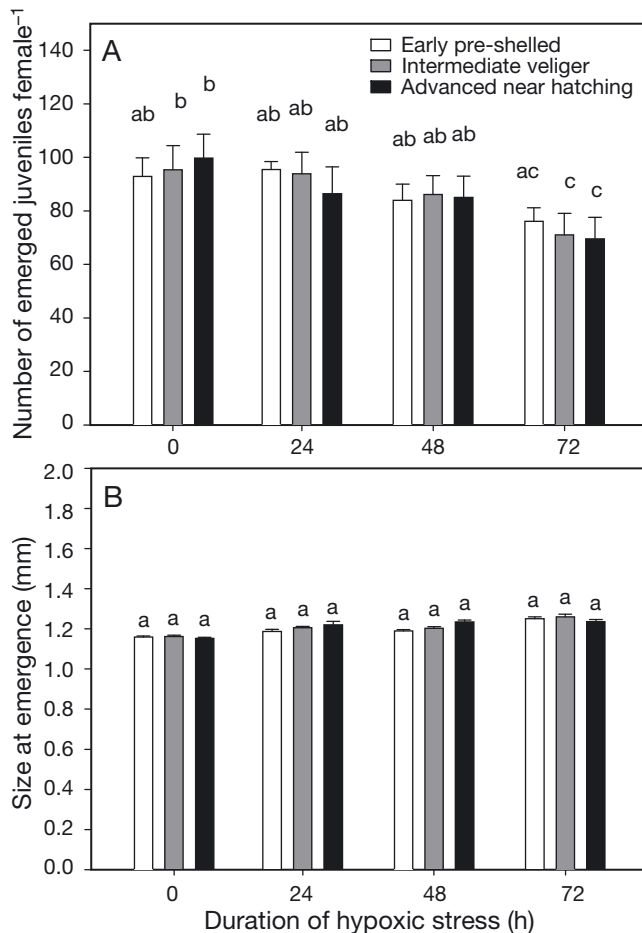


Fig 6. *Crepipatella dilatata*. Influence of early exposure to hypoxic stress on (A) the mean number of juveniles at juvenile emergence per female ($N = 325$), and (B) the mean emergence size of juveniles ($N = 325$). Vertical bars represent SE. Different letters above the bars represent significant differences

pendent of the stage of development at which embryos had been exposed to the stress (Fig. 6A, Table 1). The average number of juveniles emerging per female following 72 h of stress decreased from 93 in the controls to 76, from 95 to 71, and from 99 to 69 for early embryos, intermediate veligers, and advanced pre-hatching juveniles, respectively (Fig. 6A).

On the other hand, mean juvenile size at emergence was essentially the same following all treatments and was not affected by developmental stage during exposure or duration of the stress (Fig. 6B, Table 1). Juveniles emerged at shell lengths of about 1.2 mm in all treatments, including the control (Fig. 6B).

Other clear latent effects were also observed. Juveniles that had been stressed for a longer time during embryonic development were significantly smaller 30 d after emergence (Fig. 7A, Table 1) than were control individuals, regardless of when in develop-

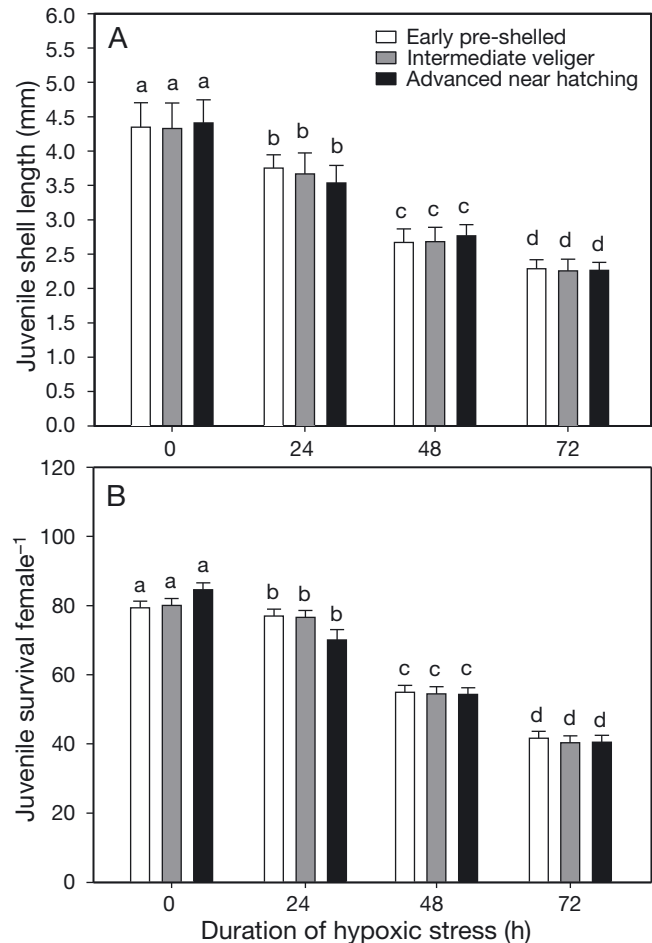


Fig 7. *Crepipatella dilatata*. Influence of early exposure to hypoxic stress on (A) mean juvenile shell length ($N = 325$) 30 d after emergence from the mother's pallial cavity, and (B) juvenile survival at 30 d after emergence from the mother's pallial cavity ($N = 325$). Embryos had been exposed to hypoxia for up to 72 h at different stages of development, as indicated, while being brooded by the mother. Vertical bars represent SE. Different letters above the bars represent significant differences

ment they had experienced hypoxia (Fig. 7A, Table 1). The largest differences were recorded between control individuals and those that had been stressed for 72 h, with final mean shell lengths of 4.3 and 2.2 mm, respectively (Fig. 7A), implying a significant and dramatic decrease in mean daily juvenile growth rates from 0.1 to only 0.03 mm d⁻¹, independent of the developmental stage at which encapsulated embryos had experienced the stress.

Longer exposure to hypoxic stress also significantly reduced juvenile survival over the 30 d monitoring period (Fig. 7B, Table 1). This was true regardless of when in development the embryos had been exposed to hypoxia (Fig. 7B, Table 1). Control females that were not exposed to hypoxic events contributed

on average approximately 80 living juveniles to the next generation at the end of the 30 d monitoring period. In contrast, females that had been exposed to hypoxia for 72 h while incubating their embryos each contributed, on average, only half as many juveniles by the end of the 30 d observation period (Fig. 7B). The extent of juvenile mortality varied with the duration of the stress that had been received during incubation, increasing from about 14% for control females to about 40% when brooding mothers had been stressed by severe hypoxia for 72 h.

DISCUSSION

Previous studies have demonstrated that brooding females of *Crepidatella dilatata* isolate the pallial cavity under environmental stress, altering conditions in the pallial fluid and impacting the brooded embryos (Chaparro et al. 2009a). In this situation, embryos are exposed simultaneously to several accumulated stressors in the pallial fluid, including excretory products, increased acidity, elevated CO₂ concentration, and hypoxia. All of these factors can act synergistically, potentially producing both immediate effects and severe latent effects (reviewed by Pechenik 2006) on offspring. In particular, isolated embryos experience a severe and long-term reduction in dissolved oxygen concentration in the fluid bathing their thin-walled egg capsules—from ~8 to <1 mg of O₂ l⁻¹, depending on the isolation time and the stage of development at which the brooded embryos are exposed (Segura et al. 2010)—when their mothers close themselves off from the external environment in response to declining external salinity. In this study, we found that severely reduced oxygen availability inside the mother's brood chamber for as little as 24 h (the shortest stress period tested) caused a number of immediate and long-term consequences for *C. dilatata* that are likely to reduce fitness considerably. Many of the brooding females in our study evicted some of their egg capsules following exposure to hypoxia, as previously reported for other calyptraeid species by Hoagland (1986), decreasing the number of juveniles eventually released per female by as much as about 25%, in comparison to the numbers released by control females; such behavior will substantially decrease reproductive fitness.

Intriguingly, the brooding mothers in our study were significantly more likely to evict their egg capsules if they had been brooding advanced embryos at the time of the stress, including those close to hatching. This may reflect the increased

metabolic demand of late-stage veligers reported for this species by Segura et al. (2010) as well as for some other gastropod species (e.g. *Fusitriton oregonensis*, Brante 2006; *Crepidula fornicata*, *C. coquimbensis*, Brante et al. 2008).

Selecting egg capsules containing late-stage embryos for eviction may reduce stress on the mother, by reducing oxygen consumption within the mantle cavity where the embryos are brooded (Brante et al. 2009, Chaparro et al. 2009a), thereby reducing the oxygen debt that must eventually be repaid (Maxime et al. 2000, Pinz & Pörtner 2003, Lewis et al. 2007). On the other hand, of course, although this behavior likely reduces the costs of additional brooding, it likely also causes the death of the evicted embryos, since, without female protection, the egg capsules will typically experience attacks by protozoa and bacteria (Hoagland 1986), reducing embryo viability (Cancino et al. 2003). The evicted, thin-walled egg capsules will also be vulnerable to predation by crustaceans and other local predators and will experience the full impact of salinity fluctuation in the estuary, with high probabilities of death caused by osmotic stress (Chaparro et al. 2008b). The consequences will thus be a pronounced reduction in realized fecundity.

Although capsule eviction should reduce fitness in this species considerably, we also noted that females of *C. dilatata* rarely evicted all of their egg capsules, particularly for the shortest stress period that we tested (24 h, Fig. 3). This behavior may be adaptive: although egg capsule eviction reduces the female's realized fecundity, it may correspondingly increase the likelihood that at least some of the offspring remaining in the brood chamber will survive to hatching.

The number of encapsulated embryos varies considerably among egg capsules in *C. dilatata*, even within a single egg mass (Segura et al. 2010). Our results show the novel and remarkable finding that females of this species are apparently able to selectively evict capsules containing different numbers of embryos. For relatively short periods of hypoxic stress (24 h in this study), females tended to preferentially evict capsules containing fewer embryos compared to their actions after being exposed to stress for 48 or 72 h (Fig. 4). It may be that egg capsules in different parts of the egg mass contain different numbers of embryos and that females are simply preferentially evicting egg capsules in some parts of the egg mass before others. Although it is not clear how females are making the distinction, the impact of preferentially evicting capsules containing fewer embryos could be beneficial in allowing females to

continue with the reproductive process when facing short-term adverse environmental conditions by selectively eliminating a smaller number of embryos initially. If the stress then dissipated, the remaining embryos could continue developing, although there would still be an overall decline in the number of descendants per female, which would likely impact population structure and dynamics (Jørgensen 1980, Rosenberg et al. 1983, Sousa 1984).

Prolonged hypoxia also extended the brooding period of *C. dilatata* considerably, in proportion to the degree of stress received. Brooding time to juvenile release was increased by at least 100% for females brooding early embryos and by about 200% for those brooding advanced embryos close to hatching. Such increases in brooding periods have also been described for a number of other brooding species in response to reduced oxygen availability (e.g. *Melanoclamys diomedea*, *Haminaea vesicula*, Cohen & Strathmann 1996; *Crepidula coquimbensis*, Brante et al. 2008). It is likely that reduced oxygen availability for embryos plays an important role in reducing rates of embryonic development (Cancino et al. 2003). In addition, the accumulation of CO₂ in the mantle cavity during periods of isolation increases acidity within the brood chamber (Chaparro et al. 2009a), thereby further inhibiting, through decalcification of the protoconch, the growth of brooded embryos (Montory et al. 2009). Although juveniles emerged from their mothers at similar sizes following all treatments in this study, brooding embryos for a longer time without increasing the number of nurse eggs available as a food source could decrease per-individual energy content at emergence, something that could then reduce juvenile survival and the effective incorporation of individuals into the adult population (Chaparro et al. 2012). In our experiments, the slower growth and higher mortality recorded over the 30 d after emergence for those juveniles that had been stressed the most as embryos appears concordant with this idea. This could be examined further in future studies.

Surprisingly, we found no effect of hypoxic stress, at any level, on average juvenile size at emergence, a finding also reported for the polychaete *Hydroides elegans* (Leung et al. 2013). This suggests that emergence in these species may somehow be triggered when embryos reach a particular size threshold (Leung et al. 2013). Further studies will be required to determine if size at hatching from the egg capsules within the mother's brood chamber is also size dependent.

Juveniles entering the adult population following extended periods of severe hypoxia showed a num-

ber of detrimental effects well after the stress had ended. These 'latent effects' (reviewed by Pechenik 2006) were revealed in our study as substantially lower post-emerging juvenile growth rates and substantially lower juvenile survival (see below), especially for individuals that had been exposed to hypoxia for the longest periods during incubation. The most severe stress (72 h) in our study, for example, reduced post-emergence juvenile shell growth rates by about 50% when compared to the growth of control juveniles, regardless of the stage of development that embryos had been at when exposed to the stress. It is not clear from our data whether the reduced growth rates were due to differential mortalities (see below) for large and small juveniles or to latent effects on individual rates of feeding or assimilation. Future studies could clarify the cause of the dramatically different juvenile growth rates that we observed. In any event, such greatly reduced growth rates would likely increase vulnerability of juveniles to predation after emergence, to the extent that smaller individuals are more vulnerable to predators than larger individuals (Gosselin & Qian 1996, 1997, Hunt & Scheibling 1998)

Juvenile mortality during the first 30 d after being released into the environment also varied in proportion to the number of hours that brooded embryos had previously been exposed to stress, and again was not affected by the stage of development at which embryos had been exposed to the stress. Whereas control juveniles showed a 30 d mortality of only approximately 15 to 20%, about 3 times as many juveniles that had been exposed to hypoxia for 72 h as brooded embryos died during the same time period. Such a high level of juvenile mortality would likely have a strong impact on population structure in the field, particularly as *C. dilatata* lacks a dispersive larval stage that could facilitate input of individuals from other populations. The cause of this mortality remains to be determined.

Prolonged exposure of brooded embryos to hypoxia will clearly impact later recruitment to the adult population, through effects on egg capsule eviction, juvenile emergence times, pre-emergence mortality, juvenile mortality, and juvenile growth rates. Consequently, long periods of low salinity in the Quemillén estuary could well lead to marked reductions in the recruitment of the early stages of *C. dilatata*, impacting the population structure of this species through both immediate and latent effects of hypoxic stress on brooded embryos. The extent to which such latent effects—rather than genetic differences—account for the observed variation in juvenile

growth rates documented in natural populations of this (e.g. Chaparro et al. 2005b) or any other organism remains to be determined.

The capacity for latent effects — effects that result from stress early in development but that first appear long after a stress has ended (e.g. after metamorphosis) — appears to be widespread among animals from a variety of phyla (reviewed by Pechenik 2006). However, responses differ among species. For example, exposing larvae to low-salinity stress reduced juvenile growth rates for the barnacle *Balanus amphitrite* (Thiyagarajan et al. 2007) but had no effect on post-metamorphic development of 3 species of calyptraeid gastropods (Diederich et al. 2011). These gastropods are not immune from latent effects; however, short periods of food deprivation early in the development of some of the same species compromised juvenile growth rates after hatching (Pechenik et al. 1996a,b, Chiu et al. 2008). As shown in this study and in that of Li & Chiu (2013), latent effects may be part of the normal response when embryos and larvae are exposed to hypoxia, in at least some species. Future studies should consider this possibility when investigating the consequences of oxygen stress on the developmental stages of other organisms.

As hypoxic conditions have become more common in coastal environments around the world, due to climate change and increasing input of anthropogenic nutrients and organic matter (Wu 2002, Levin 2003, Diaz & Rosenberg 2008, Zhang et al. 2013) and are expected to become even more common in the future (Wu 2002), interest in understanding the potential effects on ecosystems and ecosystem components has increased. Studies to date have focused on documenting molecular responses (reviewed by Wu 2002), community responses in field populations (Nilsson & Rosenberg 1994, 2000, Seitz et al. 2009), adult behavioral responses (Wu 2002, Long et al. 2008), impact on the ability of adults to protect themselves against predators (Wang et al. 2012), and impact on embryonic and larval survival (Eerkes-Medrano et al. 2013, Leung et al. 2013). Our studies with *C. dilatata* suggest that exposing developing embryos to hypoxic conditions for even short periods may have equally important, and more subtle, effects on populations dynamics mediated through elevated pre-hatching mortality, juvenile mortality, and depressed rates of juvenile growth. Future studies might also consider the possible impact on time to maturity and adult fecundity in selected species. Finally, the egg masses of some non-brooding marine polychaete and gastropod species seem to strongly

limit the supply of oxygen to the embryos developing within them (Strathmann & Strathmann 1995, Cohen & Strathmann 1996). Such embryos might be particularly susceptible to the increasing prevalence of environmental hypoxia in coastal ecosystems and potentially display latent effects in response to such stress.

Acknowledgements. The authors thank the staff at the Quempillén field station and the Calfuco Laboratory (Universidad Austral de Chile), where parts of the research were carried out. This research was funded by the grant Fondecyt-Chile 1100335 (Fondo Nacional de Investigación Científica y Tecnológica, Chile) given to O.R.C.

LITERATURE CITED

- Baeza JA, Fernández M (2002) Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. *Funct Ecol* 16:241–251
- Berger VJ, Kharazova AD (1997) Mechanisms of salinity adaptations in marine molluscs. *Hydrobiologia* 355: 115–126
- Blake JA, Grassle JP, Eckelbarger KJ (2009) *Capitella teleta*, a new species designation for the opportunistic and experimental *Capitella* sp. I, with a review of the literature for confirmed records. *Zoosymposia* 2:25–53
- Brante A (2006) An alternative mechanism to reduce intracapsular hypoxia in oviducles of *Fusitriton oregonensis* (Gastropoda). *Mar Biol* 149:269–274
- Brante A, Fernández M, Viard F (2008) Effect of oxygen conditions on intracapsular development in two calyptraeid species with different modes of larval development. *Mar Ecol Prog Ser* 368:197–207
- Brante A, Fernández M, Viard F (2009) Limiting factors to encapsulation: the combined effects of dissolved protein and oxygen availability on embryonic growth and survival of species with contrasting feeding strategies. *J Exp Biol* 212:2287–2295
- Cancino JM, Gallardo JA, Torres FA (2003) Combined effects of dissolved oxygen concentration and water temperature on embryonic development and larval shell secretion in the marine snail *Chorus giganteus* (Gastropoda: Muricidae). *Mar Biol* 142:133–139
- Castanos C, Pascual MS, Agulleiro I, Zampatti E, Elvira M (2005) Brooding pattern and larval production in wild stocks of the puelche oyster, *Ostrea puelchana* D'orbigny. *J Shellfish Res* 24:191–196
- Chaparro OR, Paschke KA (1990) Nurse egg feeding and energy balance in embryos of *Crepidula dilatata* (Gastropoda: Calyptraeidae) during intracapsular development. *Mar Ecol Prog Ser* 65:183–191
- Chaparro OR, Oyarzún RF, Vergara A, Thompson RJ (1999) Energy investment in nurse eggs and egg capsules in *Crepidula dilatata* Lamarck (Gastropoda, Calyptraeidae) and its influence on the hatching size of the juvenile. *J Exp Mar Biol Ecol* 232:261–274
- Chaparro OR, Saldivia CL, Paschke KA (2001) Regulatory aspects of the brood capacity of *Crepidula fecunda*, Gallardo 1979 (Gastropoda: Calyptraeidae). *J Exp Mar Biol Ecol* 266:97–108
- Chaparro OR, Saldivia CL, Pereda SV, Segura CJ, Montiel

- YA, Collin R (2005a) The reproductive cycle and development of *Crepidatella fecunda* (Gastropoda: Calyptraeidae) from southern Chile. *J Mar Biol Assoc UK* 85: 157–161
- Chaparro OR, Montiel YA, Cubillos VM (2005b) Direct intracapsular development: implications for feeding mechanisms in early juveniles of the gastropod *Crepidula dilatata*. *J Mar Biol Assoc UK* 85:163–169
- Chaparro OR, Segura CJ, Montiel YA, Thompson RJ, Navarro JM (2008a) Variations in the quantity and composition of seston from an estuary in southern Chile on different temporal scales. *Estuar Coast Shelf Sci* 76: 845–860
- Chaparro OR, Cubillos VM, Montiel YA, Paschke KA, Pechenik JA (2008b) Embryonic encapsulation and maternal incubation: requirements for survival of the early stages of the estuarine gastropod *Crepidatella dilatata*. *J Exp Mar Biol Ecol* 365:38–45
- Chaparro OR, Segura CJ, Montory JA, Navarro JM, Pechenik JA (2009a) Brood chamber isolation during salinity stress in two estuarine mollusk species: from a protective nursery to a dangerous prison. *Mar Ecol Prog Ser* 374:145–155
- Chaparro OR, Montory JA, Segura CJ, Pechenik JA (2009b) Effect of reduced pH on shells of brooded veligers in the estuarine bivalve *Ostrea chilensis* Philippi 1845. *J Exp Mar Biol Ecol* 377:107–112
- Chaparro OR, Schmidt AJ, Pardo LM, Andrade PV, Wagner CE, Cubillos VM (2011a) Reproductive strategy of the semelparous clam *Gaimardia bahamondei* (Bivalvia, Gaimardiidae). *Invertebr Biol* 130:49–59
- Chaparro OR, Montory JA, Pechenik JA, Cubillos VM, Navarro JM, Osoreo SJ (2011b) Ammonia accumulation in the brood chamber of the estuarine gastropod *Crepidatella dilatata*: How big a problem for mothers and brooded embryos? *J Exp Mar Biol Ecol* 410:29–38
- Chaparro OR, Lincoqueo LA, Schmidt AJ, Veliz D, Pechenik JA (2012) Comparing biochemical changes and energetic costs in gastropods with different developmental modes: *Crepidatella dilatata* and *C. fecunda*. *Mar Biol* 159:45–56
- Chiu JMY, Wang H, Thiagarajan V, Qian PY (2008) Differential timing of larval starvation effects on filtration rate and growth in juvenile *Crepidula onyx*. *Mar Biol* 154: 91–98
- Christensen AB, Colacino JM (2000) Respiration in the burrowing brittlestar, *Hemipholis elongata* Say (Echinodermata, Ophiuroidea): a study of the effects of environmental variables on oxygen uptake. *Comp Biochem Physiol A Mol Integr Physiol* 127:201–213
- Cohen C, Strathmann RR (1996) Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. *Biol Bull* 190: 8–15
- Connor KM, Gracey AY (2012) High-resolution analysis of metabolic cycles in the intertidal mussel *Mytilus californianus*. *Am J Physiol Regul Integr Comp Physiol* 302: R103–R111
- Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321:926–929
- Diederich SM, Jarrett JN, Chaparro OR, Segura CJ, Arelano SM, Pechenik JA (2011) Low salinity stress experienced by larvae does not affect post-metamorphic growth or survival in three calyptraeid gastropods. *J Exp Mar Biol Ecol* 397:94–105
- Djangmah JS, Shumway SE, Davenport J (1979) Effects of fluctuating salinity on the behaviour of the West African blood clam *Anadara senilis* and on the osmotic pressure and ionic concentrations of the haemolymph. *Mar Biol* 50:209–213
- Eerkes-Medrano D, Menge BA, Sislak C, Langdon CJ (2013) Contrasting effects of hypoxic conditions on survivorship of planktonic larvae of rocky intertidal invertebrates. *Mar Ecol Prog Ser* 478:139–151
- Gallardo CS (1979) Especies gemelas del genero *Crepidula* (Gastropoda, Calyptraeidae) en la costa de Chile; una redescription de *C. dilatata* Lamarck y descripción de *C. fecunda* n.sp. *Stud Neotrop Fauna Environ* 14:215–226
- Gillespie JM, McClintock JB (2007) Brooding in echinoderms: How can modern experimental techniques add to our historical perspective?. *J Exp Mar Biol Ecol* 342: 191–201
- Gorr TA, Wichmann D, Hu J, Hermes-Lima M and others (2010) Hypoxia tolerance in animals: biology and application. *Physiol Biochem Zool* 83:733–752
- Gosselin LA, Qian PY (1996) Early post-settlement mortality of an intertidal barnacle: a critical period for survival. *Mar Ecol Prog Ser* 135:69–75
- Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146:265–282
- Hand SC, Stickle WB (1977) Effects of tidal fluctuations of salinity on pericardial fluid composition of the American oyster *Crassostrea virginica*. *Mar Biol* 42:259–271
- Hendler G (1975) Adaptational significance of the patterns of ophiuroid development. *Am Zool* 15:691–715
- Ho DH, Burggren WW (2012) Parental hypoxic exposure confers offspring hypoxia resistance in zebrafish (*Danio rerio*). *J Exp Biol* 215:4208–16, doi:10.1242/jeb.074781
- Hoagland KE (1986) Patterns of encapsulation and brooding in the Calyptraeidae (Prosobranchia: Mesogastropoda). *Am Malacol Bull* 4:173–183
- Howarth R, Chan F, Conley DJ, Garnier J, Doney SC, Marino R, Billen G (2011) Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Front Ecol Environ* 9:18–26
- Hunt HL, Scheibling RE (1998) Spatial and temporal variability of patterns of colonization by mussels (*Mytilus trossulus*, *M. edulis*) on a wave-exposed rocky shore. *Mar Ecol Prog Ser* 167:155–169
- Ituarte C (2009) Unusual modes of oogenesis and brooding in bivalves: the case of *Gaimardia trapesina* (Mollusca: Gaimardiidae). *Invertebr Biol* 128:243–251
- Jones JC, Reynolds JD (1999) Oxygen and the trade-off between egg ventilation and brood protection in the common goby. *Behaviour* 136:819–832
- Jørgensen BB (1980) Seasonal oxygen depletion in the bottom waters of a Danish fjord and its effects on the benthic community. *Oikos* 34:68–76
- Leung YS, Shin PKS, Qiu JW, Ang PO, Chiu JMY, Thiagarajan V, Cheung SG (2013) Physiological and behavioural responses of different life stages of a serpulid polychaete to hypoxia. *Mar Ecol Prog Ser* 477:135–145
- Levin LA (2003) Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanogr Mar Biol Annu Rev* 41:1–45
- Lewis JM, Costa I, Val AL, Almeida-Val VMF, Gamperl AK, Driedzic WR (2007) Responses to hypoxia and recovery: repayment of oxygen debt is not associated with compensatory protein synthesis in the Amazonian cichlid, *Astronotus ocellatus*. *J Exp Biol* 210:1935–1943

- Li A, Chiu JMY (2013) Latent effects of hypoxia on the gastropod *Crepidula onyx*. *Mar Ecol Prog Ser* 480:145–154
- Liu CC, Chiu JMY, Li L, Shin PKS, Cheung SG (2011) Physiological responses of two sublittoral nassariid gastropods to hypoxia. *Mar Ecol Prog Ser* 429:75–85
- Long WC, Brylawski BJ, Seitz RD (2008) Behavioral effects of low dissolved oxygen on the bivalve *Macoma balthica*. *J Exp Mar Biol Ecol* 359:34–39
- Matear RJ, Hirst AC (2003) Long-term changes in dissolved oxygen concentrations in the ocean caused by protracted global warming. *Global Biogeochem Cycles* 17:1125, doi: 10.1029/2002GB001997
- Maxime V, Pichavant K, Boeuf G, Nonnotte G (2000) Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. *Fish Physiol Biochem* 22:51–59
- McMahon BR (2001) Respiratory and circulatory compensation to hypoxia in crustaceans. *Respir Physiol* 128: 349–364
- Middelburg JJ, Levin LA (2009) Coastal hypoxia and sediment biogeochemistry. *Biogeosciences* 6:1273–1293
- Miller SE (1993) Larval period and its influence on post-larval history: comparison of lecithotrophy and facultative planktotrophy in the aeolid nudibranch *Phestilla sibogae*. *Mar Biol* 117:635–645
- Montroy JA, Chaparro OR, Cubillos VM, Pechenik JA (2009) Isolation of incubation chambers during brooding: effect of reduced pH on protoconch development in the estuarine gastropod *Crepipatella dilatata* (Calyptraeidae). *Mar Ecol Prog Ser* 374:157–166
- Moran AL (2000) Calcein as a marker in experimental studies newly-hatched gastropods. *Mar Biol* 137:893–898
- Nilsson HC, Rosenberg R (1994) Hypoxic response of two marine benthic communities. *Mar Ecol Prog Ser* 115: 209–217
- Nilsson HC, Rosenberg R (2000) Succession in marine benthic habitats and fauna in response to oxygen deficiency: analysed by sediment profile-imaging and by grab samples. *Mar Ecol Prog Ser* 197:139–149
- Pechenik JA (2006) Larval experience and latent affects — metamorphosis is not a new beginning. *Integr Comp Biol* 46:323–333
- Pechenik JA, Rice ME (2001) Influence of delayed metamorphosis on postsettlement survival and growth of the sipunculan *Apionsoma misakiana*. *Invertebr Biol* 120: 50–57
- Pechenik JA, Hammer K, Weise C (1996a) The effect of starvation on acquisition of competence and post-metamorphic performance in the marine prosobranch gastropod *Crepidula fornicata* (L). *J Exp Mar Biol Ecol* 199:137–152
- Pechenik JA, Estrella S, Hammer K (1996b) Food limitation stimulates metamorphosis and alters postmetamorphic growth rate in the marine prosobranch gastropod *Crepidula fornicata*. *Mar Biol* 127:267–275
- Pechenik JA, Wendt D, Jarrett J (1998) Metamorphosis is not a new beginning: Larval experience reduces rates of postlarval growth, development, and survival in marine invertebrates. *Bioscience* 48:901–910
- Phillips NE (2002) Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. *Ecology* 83:2562–2574
- Phillips NE (2004) Variable timing of larval food has consequences for early juvenile performance in a marine mussel. *Ecology* 85:2341–2346
- Pinz I, Pörtner HO (2003) Metabolic costs induced by lactate in the toad *Bufo marinus*: new mechanism behind oxygen debt? *J Appl Physiol* 94:1177–1185
- Qiu JW, Qian PY (1999) Tolerance of the barnacle *Balanus amphitrite amphitrite* to salinity and temperature stress: effects of previous experience. *Mar Biol* 188:123–132
- Rosenberg R, Arntz WE, Chuman de Flores E, Flores LA, Carbajal G, Finger I, Tarazona J (1983) Benthos biomass and oxygen deficiency in the upwelling system off Peru. *J Mar Res* 41:263–279
- Segura CJ, Chaparro OR, Paschke KA, Pechenik JA (2010) Capsule walls as barriers to oxygen availability: implications for the development of brooded embryos by the estuarine gastropod *Crepipatella dilatata* (Calyptraeidae). *J Exp Mar Biol Ecol* 390:49–57
- Seitz RD, Dauer DM, Llansó RJ, Long WC (2009) Broad-scale effects of hypoxia on benthic community structure in Chesapeake Bay, USA. *J Exp Mar Biol Ecol* 381:S4–S12
- Shumway SE (1977) The effect of fluctuating salinity on the tissue water content of eight species of bivalve molluscs. *J Comp Physiol* 116:269–285
- Sousa WP (1984) The role of disturbance in natural communities. *Annu Rev Ecol Syst* 15:353–391
- Strathmann RR, Strathmann MF (1995) Oxygen supply and limits on aggregation of embryos. *J Mar Biol Assoc UK* 75:413–428
- Sussarellu R, Fabioux C, Sanchez MC, Le Goïc N, Lambert C, Soudant P, Moraga D (2012) Molecular and cellular response to short-term oxygen variations in the Pacific oyster *Crassostrea gigas*. *J Exp Mar Biol Ecol* 412:87–95
- Tankersley RA, Dimock RV Jr (1993) The effect of larval brooding on the respiratory physiology of the freshwater unionid mussel *Pyganodon cataracta*. *Am Midl Nat* 130: 146–163
- Thiyagarajan V, Pechenik JA, Gosselin LA, Qian PY (2007) Juvenile growth in barnacles: combined effect of delayed metamorphosis and sub-lethal exposure of cyprids to low-salinity stress. *Mar Ecol Prog Ser* 344:173–184
- Toro JE, Winter JE (1983) Estudios en la ostricultura Quempillén, un estuario del Sur de Chile. Parte I. La determinación de los factores abióticos y la cuantificación del seston como oferta alimenticia y su utilización por *Ostrea chilensis*. *Mem Asoc Latinoam Acuicult* 5:129–144
- Vogt G (2013) Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. *Biol Rev Camb Philos Soc* 88: 81–116
- Wang Y, Hu M, Cheung SG, Shin PKS, Lu W, Li J (2012) Chronic hypoxia and low salinity impair anti-predatory responses of the green-lipped mussel *Perna viridis*. *Mar Environ Res* 77:84–89
- Wilson AB, Ahnesio I, Vincent ACJ, Meyer A (2003) The dynamics of male brooding, mating patterns, and sex roles in pipefishes and seahorses (Family Syngnathidae). *Evolution* 57:1374–1386
- Wu RSS (2002) Hypoxia: from molecular responses to ecosystem responses. *Mar Pollut Bull* 45:35–45
- Zhang J, Cowie G, Naqvi SWA (2013) Hypoxia in the changing marine environment. *Environ Res Lett* 8:1–3